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Triethyltin: Ambient Temperature Alters Visual System Toxicity

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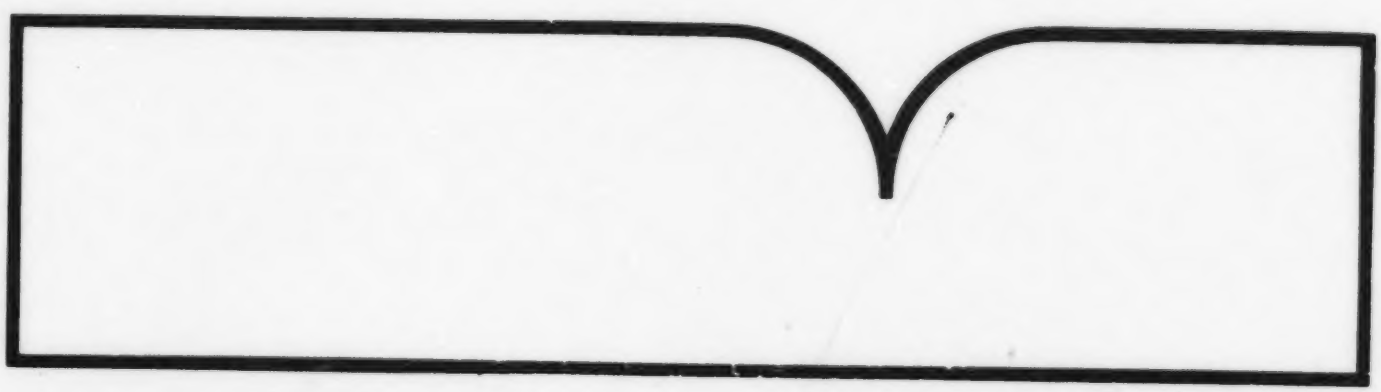

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16. ABSTRACT

Previous studies have indicated that acute exposure to triethyltin (TET) increases latencies of the flash evoked response (VER) recorded from the rat cortex. TET also produces hypothermia, which may be modified by altering environmental (ambient) temperature. In this study, the role of ambient temperature in determining the effects of acute TET upon the VER was examined. Rats with chronically implanted electrodes were administered either TET (6mg/kg) or saline, and maintained in either a warm (30°C) or cool (22°C) environment for the next 7 hrs. VERs were recorded during this 7 hr period, and at regular intervals for the next 2 weeks. TET increased VER peak latencies. VER peak latencies recorded from animals exposed to TET in a warm room remained elevated for a longer period of time, thus indicating a more sever impact of the TET exposure. This study indicates that toxicant induced alterations in core temperature are potential determinants of other toxicant-induced effects.

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Triethyltin: Ambient Temperature Alters Visual System Toxicity[1]

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DYER, R. S. AND W. E. HOWELL. *Triethyltin: Ambient temperature alters visual system toxicity*. NEUROBEHAV. TOXICOL. TERATOL. 4(2) 267–271, 1982.—Previous studies have indicated that acute exposure to triethyltin (TET) increases latencies of the flash evoked response (VER) recorded from the rat cortex. TET also produces hypothermia, which may be modified by altering environmental (ambient) temperature. In this study, the role of ambient temperature in determining the effects of acute TET upon the VER was examined. Rats with chronically implanted electrodes were administered either TET (6 mg/kg) or saline, and maintained in either a warm (30°C) or cool (22°C) environment for the next 7 hrs. VERs were recorded during this 7 hr period, and at regular intervals for the next 2 weeks. TET increased VER peak latencies. VER peak latencies recorded from animals exposed in a cool room gradually returned to pre-TET values. Latencies recorded from animals exposed to TET in a warm room remained elevated for a longer period of time, thus indicating a more severe impact of the TET exposure. This study indicates that toxicant-induced alterations in core temperature are potential determinants of other toxicant-induced effects.

Tin Organotin Alkyltin Triethyltin Neurotoxicity Core temperature Ambient temperature
Temperature-dependent toxicity Visual system toxicity Visual evoked potential Visual evoked response

TRIETHYLTIN (TET) is a highly neurotoxic compound. Numerous studies have explored the metabolic, morphological, physiological, and behavioral effects of exposure to TET (e.g., [1, 4, 6, 10, 14]). One of the most extensively studied consequences of TET exposure is CNS demyelination. Several studies have also suggested that TET produces an ambient temperature-dependent hypothermia in rats [6,12]. This finding is important, since hypothermia may account for some of the effects of TET which would otherwise be ascribed to myelinopathy. For example, Rose and Aldridge [15] demonstrated that the depressed incorporation of [^{32}P] phosphate into rat brain phospholipids which was found to occur following exposure to TET [17] was in fact a result of hypothermia, and could be prevented when hypothermia was reduced by warm environmental conditions.

Acute exposure to TET has been shown to increase latencies of the visual evoked response (VER) [9,10]. Since the optic nerve is sensitive to the demyelinating effects of TET [16], the most obvious interpretation of the data was conduction slowing secondary to demyelination. However, hypothermia also increases evoked potential latencies (e.g., [11]), probably due to the relationship between reduced temperature and reduced nerve conduction velocity [7,18]. Therefore, slowed conduction (increased latencies) of the VER might reflect either hypothermia, alterations in myelin, or both. The purpose of the present study was to (a) verify that TET does produce an ambient temperature-dependent hypothermia; and (b) assess the influence of ambient temperature upon TET-induced alterations in the VER recorded from the rat visual cortex.

METHOD

Adult male Long-Evans hooded rats were obtained from Charles River Breeding Laboratories. Except as otherwise indicated, the animals were maintained in individual plastic cages with wood chip bedding and ad lib access to food and water throughout the experiment. Seventy-eight rats were used in the core temperature study, and an additional 40 rats were used in the VER study.

Core Temperature

Three experiments were performed to assess the influence of TET upon core temperature. In all three, core temperatures were recorded using a Yellow Springs Instruments thermister type multiprobe thermometer. The rats were placed in a commercial restraining tube and the probe was inserted 75 mm into the rectum. Digital readings were recorded to the nearest 0.1°C. During the 6-hr sessions, the rats were restrained continuously and the probe remained in place. All other readings were taken with the rats placed in the restraining tube for 5 min before recordings. In the first experiment, 34 rats, kept in a room maintained at 22°C, were injected IP with either 0 (vehicle, n=14), 3(n=6), 6(n=8), or

[1]This paper has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation of use.

9(n=6) mg/kg TET bromide in saline vehicle. Core temperatures were recorded immediately before injection and every 0.5 hr thereafter for 6 hrs. The animals injected with 9 mg/kg TET also had temperatures taken 24, 48, and 72 hrs post-injection.

In the second experiment, rats were injected with either 0 (n=10) or 9 (n=10) mg/kg TET and maintained in a room at 30°C. Again, core temperatures were recorded immediately before injection and every 0.5 hr afterwards for 6 hr. The animals exposed to 9 mg/kg TET also had their temperatures recorded 138 hrs following injection.

In the third experiment, 32 rats in a room maintained at 22°C were injected with either 0 (saline vehicle, n=8), 3 (n=8), 6 (n=8), or 9 (n=8) mg/kg TET. Temperatures were recorded immediately before injection and 1, 2, 5, and 6 days later.

Visual Evoked Responses

Electrodes were implanted in rats for recording the VER, using surgical procedures described in detail elsewhere [8]. The electrodes consisted of 00–90 stainless steel screws threaded into the skull. Coordinates for placement were: visual cortex, 6 mm posterior to bregma, 3 mm lateral to the midline; reference, 2 mm anterior to bregma, 2 mm lateral to the midline, and ground, 5 mm anterior, 2 mm lateral, on the side contralateral to the visual cortex and reference electrodes. The screws were connected to an Amphenol receptacle via nichrome wires, and the receptacle was cemented to the skull using dental acrylic. Animals were then administered 100,000 units penicillin G and returned to their home cage. Recordings were begun no sooner than 1 week following surgery.

VER recordings were made in a box with mirrors on 3 walls and the strobe lamp from a Grass PS-2 photostimulator on the 4th wall. The intensity setting for the 10 μsec flash was 16, which corresponded to a peak power of 4.53×10^7 lux measured in the center of the chamber. Details of the recording chamber are available elsewhere [8]. During each recording session, the averaged VER to 64 flashes was obtained. Flashes were presented at 2-sec intervals, and recordings were obtained through AC coupled amplifiers with low and high frequency cutoffs set at 1.0 Hz and 10 kHz respectively. Averages were obtained using a Nicolet 1024 signal averager with 0.4 msec/data point resolution, and copies of the waveforms were made by a Hewlett-Packard X-Y plotter. Amplitudes and latencies of the first three positive and negative waves were obtained according to previously determined conventions [9].

The rats were tested on 5 separate days. On the first day, 1 drop of 1% atropine sulfate was placed in each eye 20 min prior to testing. An averaged VER was obtained as described above, the rat was injected with either 6 mg/kg TET (n=20) or saline vehicle (n=20). Following each VER session, the animal was immediately returned to its home cage. Additional averaged VERs were obtained 1.5, 4.0, 7.0, 24, 48, 120, and 300 hrs following the injections. Atropine was not readministered prior to the 1.5 and 4 hr sessions since it was presumed the initial treatment was still effective.

Half of each injection group was maintained and tested in a warm environment (30°C) beginning 30 min before the first test session and continuing until after the 7-hr post-injection test session. All other tests for these and other animals occurred at normal room temperature (22°C), which was also the temperature of the animal colony room. No efforts were made to record core temperature in these animals. Based upon the core temperature studies (see Results), it was assumed that the TET-exposed rats became hypothermic for at least the first 7 hrs, but that the TET-exposed rats maintained in a warm environment became considerably less hypothermic than those maintained at normal room temperature.

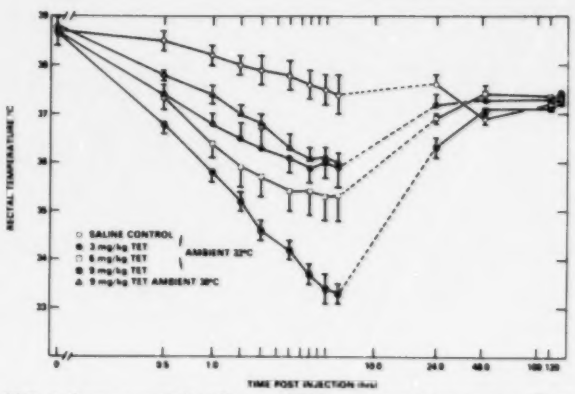

FIG. 1. Summary of the effects of TET upon core temperature. Data points are means ± SEM. Increasing ambient temperature attenuated the hypothermia produced by 9 mg/kg TET.

Statistical Analysis

All VER amplitudes and latencies were converted to difference scores from the preinjection values. A 2 (dose) × 2 (temp) × 6 (time) repeated measures ANOVA was then performed on each of the 11 variables (P1, N1, P2, N1, P3, and N3 latencies; P1N1, N1P2, P2N2, N2P3, P3N3 amplitudes). In order to approximate an experiment-wise α was set at 0.05/11 = 0.0045 for each ANOVA. To further reduce the probability of type 1 errors, only the following effects were included in the ANOVAs: Dose, Dose × Temperature, and Dose × Time × Temperature.

RESULTS

Core Temperature

Acute TET exposure reduced core temperature in a dose-dependent fashion. The data from all of the core temperature studies are summarized in Fig. 1. All animals, even controls, had slightly reduced core temperatures over the course of the experiment. The magnitude of temperature reduction was dependent upon dosage of TET, such that the rats injected with 3 mg/kg TET had temperatures about 1.5° below controls, while the rats injected with 6 and 9 mg/kg TET had core temperatures which were about 2.0° and 4.0° below controls, respectively. The TET-induced alterations in temperature were largely gone 24 hrs following injection, although the rats exposed to 9 mg/kg TET were still slightly hypothermic. Rats injected with 9 mg/kg TET and maintained in the warm (30°C) environment did not become as hypothermic as those maintained in the cool (22°C) environment. The hypothermia produced by 9 mg/kg TET in the warm environment was roughly equivalent to that produced by 3 mg/kg TET in the cool environment.

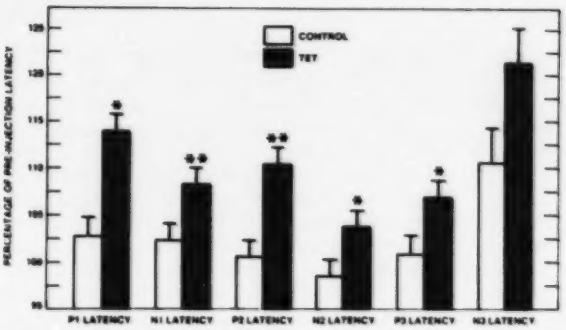

FIG. 3. Main effects of TET upon VER peak latencies. the values are means (±SEM) which have been transformed to percentage of pre-injection values for each rat, and collapsed across both post-injection hrs and temperature conditions. *=$p<0.01$, **=$p<0.0045$.

cates that the daily exposure produced a prolonged time course of latency alterations. In the present study, VER parameters of the cool TET-exposed animals had returned to baseline within 24 hrs after exposure, while in the previous study the alterations in N1 latency remained for at least 2 weeks following termination of exposure. This difference in the time course of TET toxicity may have occurred due to the higher cumulative dosage of TET required to produce these effects in the previous study (6×1.5=9 mg/kg vs 6 mg/kg).

Two important results emerge from the present study. First, it is clear that even though hypothermia produces alterations in the VER which are somewhat similar in character to those produced by TET [9], the presence of alterations in N1 latency which are similar in magnitude in both the warm and cold-exposed rats coupled with the increased duration of toxicity in warm-exposed rats argues strongly that the previous results may not be accounted for by hypothermia. Second, this study points out the temperature-dependent toxicity of TET. Even though exposure to warm environmental temperatures was of relatively short duration (7 hrs), the effects of this exposure were persistent. N1 latencies of the warm-exposed TET-injected rats only began to show signs of returning to normal 12 days following exposure. Cold-exposed TET-injected rats had normal N1 latencies 24 hrs following exposure.

The finding that reduction of toxicant-induced hypothermia increases rather than decreases the toxicity of the agent in question is not novel. Prevention of carbon monoxide-induced hypothermia significantly increases lethality at a normally non-lethal concentration of CO [3].

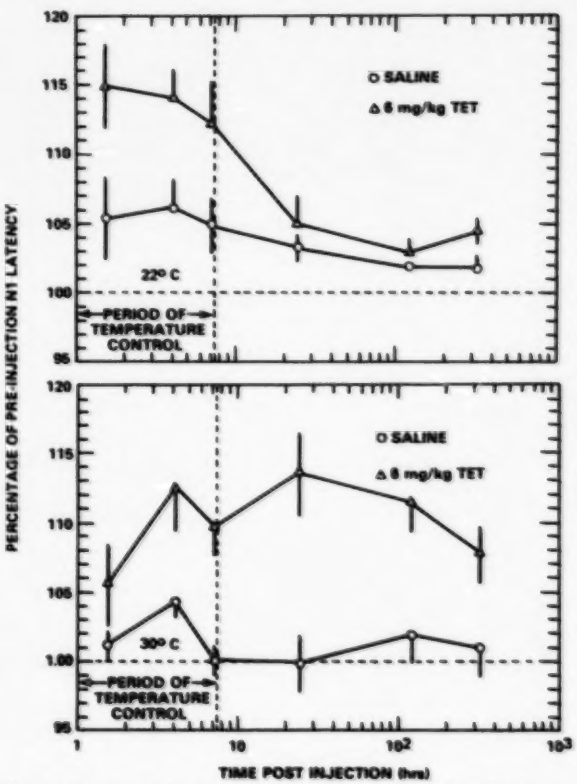

FIG. 4. Time- and temperature-dependent nature of TET effects upon the latency of VER peak N1. Top: animals maintained at "cool" (22°C) room temperature during the period of temperature control. Differences between TET and control rats were absent 24 hrs following exposure; Bottom: animals maintained in a warm (30°C) environment during the period of temperature control. Differences between TET and control rats were still evident 300 hrs following exposure.

These data provide further evidence for the critical nature of environmental temperature in the assessment of neurotoxicity.

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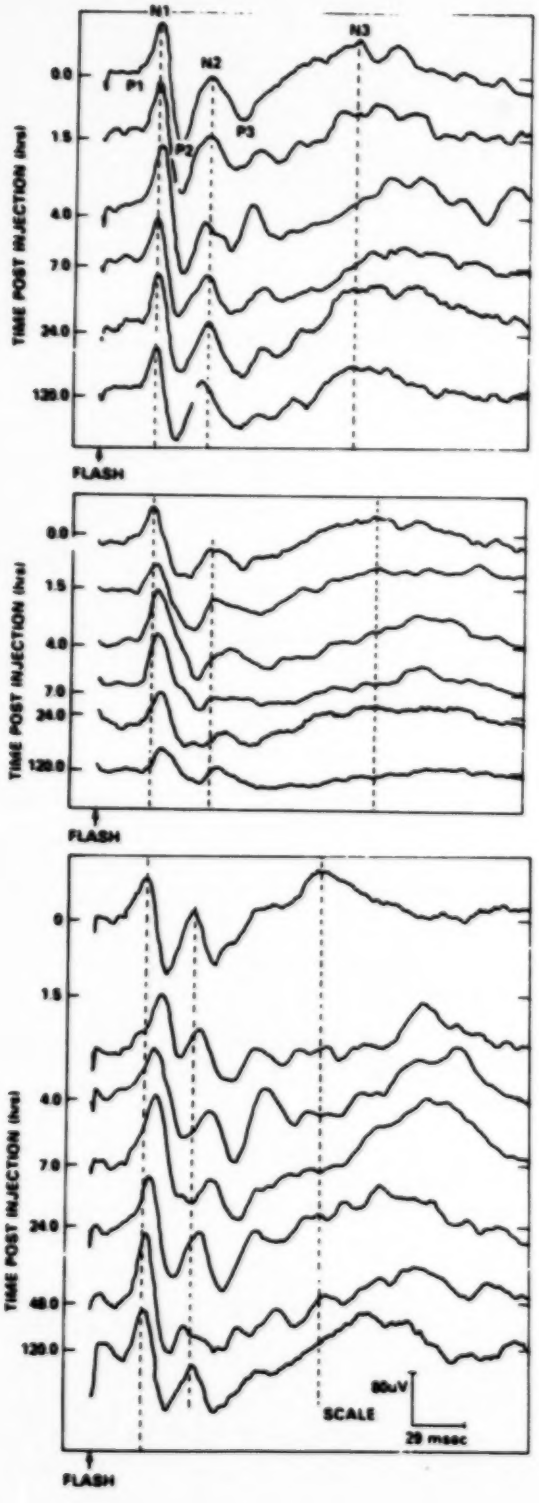

FIG. 2. Averaged flash-evoked potentials recorded from the visual cortex of one rat in each of three groups. Top: control rat, recordings taken at the times indicated on the ordinate. The peak designations used in the study are indicated on the 0.0 hr waveform. This control was injected with saline at 0.0 hrs and maintained in a 30°C environment for 7 hrs; Middle: same as above except the rat was injected with 6 mg/kg TET at 0.0 hrs; Bottom: 6 mg/kg TET-treated rat maintained at "normal" (22°C) room temperature throughout the study. Calibration applies to all 3 sets of waveforms. Dashed vertical lines are drawn through the pre-injection N1, N2, and N3 peaks.

VERs

Figure 2 illustrates the VERs recorded from TET-exposed rats in the warm and cool environments, and from a control rat in the warm environment. TET increased latencies of all peaks, although only N1 and P2 latencies differed statistically from control with $\alpha=0.0045$. Figure 3 summarizes these data. There were no statistically signficant effects of TET upon amplitudes. There were no significant dose × temperature interactions, but TET produced alterations in N1 latency which were dependent upon the temperture condition and the time of measurement (significant dose × temperature × time interaction). Rats exposed to TET in a warm room had elevated N1 latencies (compared to saline controls in the warm room) at all measurement times following injection. Rats exposed to TET in the cool room had N1 latencies which were elevated 1.5, 4 and 7 hrs following injection, but returned to control values within 24 hrs. These data are illustrated in Fig. 4.

DISCUSSION

These studies confirm that TET produces hypothermia which can be at least partially attenuated by maintenance in a 30°C environment. Even control (saline injected) animals exhibited a slight drop in temperature over the course of the experiment. This drop may reflect recovery from an initial hyperthermia in all rats induced by the stress of being placed in the restraining tube [13]. The failure to observe this hyperthermia in any rats after the first day probably reflects habituation to the restraining tube.

The failure to completely prevent hypothermia from developing by maintaining animals exposed to 9 mg/kg TET in a 30°C environment was surprising, since this environmental temperature is effective in preventing the more severe hypothermia which occurs during exposure to either 1000 ppm carbon monoxide or hypoxic hypoxia [3]. It had been presumed that the hypothermia induced by exposure to TET reflected the histotoxic hypoxia which this agent is known to produce by virtue of its uncoupling of oxidative phosphorylation [1]. These data suggest that other mechanisms may also be involved.

The VER study produced several significant findings. First, it replicated the findings of Dyer and Howell [9] in that TET produced increased VER latencies in the absence of major effects upon VER amplitudes. Unlike the previous study, TET was administered in a single injection and the time course of alterations was studied. Comparing the TET-exposed animals kept in the cool environment in the present study with the high dose animals exposed to TET daily in the previous study (which was done at about the same environmental temperature as the cool exposure of this study), indi-

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